SALINITY AND ITS EFFECTS ON THE PHYSIOLOGICAL RESPONSE OF BEAN (PHASEOLUS VULGARIS L.)

ЕФЕКТ НА ЗАСОЛЯВАНЕТО ВЪРХУ ФИЗИОЛОГИЧНИЯ ОТГОВОР НА ФАСУЛА (PHASEOLUS VULGARIS L.)

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ABSTRACT

The effect of salt stress on the physiological reaction in young bean plants was studied. The plants were grown in pots as hydroponic cultures in half-strength Hoagland nutrient solution under controlled conditions in a climatic room. The plants were treated for 7 days with NaCl and Na_2SO_4 (concentration 100 mM), starting at the appearance of the first trifoliate leaf unfolded. The salts were added to the nutrient solution.

It was established that the equimolar concentrations of both salt types caused stress in the young bean plants, which found expression in the suppression of growth, photosynthesis activity and caused changes in stomata status (conductivity, number and size). The transpiration and the cell water potential in salt-treated plants were reduced. The MDA level in root and shoot, and the proline content was increased.

Key words: bean, salinity, salt stress, growth, leaf-gas exchange, water potential, stomata number and size.

РЕЗЮМЕ

Проучен беше ефектът от солевия стрес върху физиологичната реакция на млади растения фасул. Растенията бяха отгледани в съдове като хидропонни култури в ½ хранителен разтвор на Хогланд в климатичен бокс при контролирани условия. След оформянето на първия троен лист растенията бяха третирани с еквимоларни разтвори от NaCl и Na_2SO_4 (концентрация 100 mM),. Солите бяха добавени към хранителния разтвор.

Установено беще, че еквимоларните концентрации от двата вида соли предизвикват стрес в младите растения фасул, който се изразява в подтискане на растежа и фотосинтетичната активност, и промяна в устичното състояние (проводимост, брой и размери). Транспирацията и водния потенциал на клетките в засолените растения бяха понижени. Отчетено беше повишаване количеството на пролина и нивото на липидната пероксидация както в корените, така и в надземните части на растенията.

Ключови думи: фасул, засоляване, солеви стрес, листен газообмен, воден потенциал, брой и размери на устицата.

INTRODUCTION

The salinity of soil is a widespread environmental problem and important factor in limiting agricultural productivity. Salinity affects plants through osmotic effects, ion-specific effects and oxidative stress [19]. The high salinity levels of soil and irrigation water are known to affect many physiological and metabolic processes, leading to cell growth reduction [8]. The effect of excess salinity markedly decrease the growth and transpiration rates [20], photosynthesis and pigment contents [24,25]. High exogenous salt concentrations affect seed germination, water deficit, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress [14, 15].

The effect of salinity stress on photosynthetic rate and water use efficiency was closely related to leaf anatomical features. The reduction of mesophyl conductance was associated with leaf thickness and smaller intercellular spaces in the mesophyl of salt-stressed leaves, which may have made the part towards the sites of CO_2 fixation more difficult [23].

Osmotic effects are due to salt-induced decrease in the soil water potential [13]. Salt stress induces cellular accumulation of damaging active oxygen species (ROS). ROS are highly reactive and toxic to plants and can lead to cell death by causing damage to proteins, membrane lipids [15], and nucleic acids [21].

Saline environments are generally correlated with changes in plant lipid metabolism [8]. Lipid peroxidation has been associated with damages provoked by a variety of environmental stresses. Lipid peroxidation, induced by free radicals, is also important in membrane deterioration [8, 11, 15, 17].

A large number of plants species accumulate proline (Pro) in response to salinity stress and that accumulation may play a role in combating salinity stress [2, 18, 19]. However, data do not always indicate a positive correlation between the osmolyte accumulation and adaptation to stress [16].

In the present work, the effects of salt treatment on lipid peroxidation, water potential and water content of bean were studied. Leaf gas-exchange and leaf anatomy were also analyzed.

MATERIAL AND METHODS

Bean seeds (Phaseolus vulgaris L.,), cultivar Gina, were surface-sterilized with a 0.5% NaOCL (sodium hypochlorite) solution for 1 min and then washed thoroughly in sterilized water. The seeds were germinated at dark at 26^0C in vermiculite media. After that, they were transferred in pots filled by half-strength Hoagland nutrient solution and grown in a growth chamber under controlled environmental conditions. The conditions, maintained during the experiments, were the following: light duration – 14 hours, light intensity (PAR) 250 μmol m^{-2} s^{-1}, temperature – 22 ± 2 °C and relative air humidity – 60 ± 5%. At the appearance of the first trifoliate leaf, an experimental design with three treatments was arranged for every cultivar:

control – plants, supplied by ½ of Hoagland solution;

plants, supplied by ½ of Hoagland solution enriched with 100 mM NaCl;

plants, supplied by ½ of Hoagland solution enriched with 100 mM Na_2SO_4;

The treatment of plants with salts continued for 7 days.

Lipid peroxidation was measured as the amount

Table 1. Effect of salinity on the leaf gas-exchange parameters in bean plants.

Young bean plants are subjected to salt stress by adding 100 mM of NaCl and Na_2SO_4 to the Hoagland nutrient solution. P_N – Net photosynthetic rate [μmol (CO_2) m^{-2} s^{-1}]; E – Transpiration rate [mmol (H_2O) m^{-2} s^{-1}]; g_s – stomata conductance [mol m^{-2} s^{-1}]; P_N /E ratio – water use efficiency ; and LA – leaf aria (cm^2); values are mean ±SE. * <0.5 ** <0.1

Таблица 1. Ефект от засоляването върху параметрите на листния газообмен на фасула. Млади растения фасул бяха отгледани в условия на солеви стрес чрез добавяне на 100 mM NaCl Na_2SO_4 към хранителния разтвор. . P_N – Скорост на фотосинтезата [μmol (CO_2) m^{-2} s^{-1}]; E – Скорост на транспирацията [mmol (H_2O) m^{-2} s^{-1}]; g_s – устична проводимост [mol m^{-2} s^{-1}]; P_N /E – ефективност на използваната вода ; and LA – листна площ (cm^2);). Стойностите са осреднени ±SE * <0.5 ** <0.1

Parameters	Control	100 mM NaCL	100 mM Na_2SO_4
P_N	5.25±0.69	1.61±0.01**	1.36±0.05**
E	2.25±0.05	0.95±0.03**	0.82±0.02**
g_s	0.062±0.002	0.031±0.001	0.021±0.003**
P_N /E	2.33±0.15	1.69±0.20	1.65±0.19
LA (cm^2)	259±7.4	227±8.2*	202±2.6**

Table 2. Anatomical characteristics of leaves of Bean exposed for 7 days to 100 mM NaCL and Na_2SO_4; values are mean ±SE.

Таблица 2. Анатомична характеристика на листа от фасул след седемдневно засоляване с 100 mM NaCL и Na_2SO_4.). Стойностите са осреднени ±SE.

Parameters	Control	100 mM NaCL	100 mM Na_2SO_4
Upper epidermis			
Number of stomata	507,6 ± 14,37	782,8 ± 16,49	722,8 ± 13,17
Stomata lenght(µm)	22,97 ± 0,32	15,87 ± 0,58	20,76 ± 0,74
Stomata width (µm)	18,38 ± 0,13	11,77 ± 0,6	15,34 ± 0,48
Lower epidermis			
Number of stomata	75,2 ± 4,22	106,8 ± 5,59	182,8 ± 9,25
Damaged	0	145.2±8.26	29.6±6.39
Stomata lenght (µm)	27,85 ± 0,49	21,43 ± 0,38	24,63 ± 0,53
Stomata width (µm)	19,6 ± 0,25	18,2 ± 0,42	17,79 ± 0,43

of thiobarbituric acid reactive substance (TBARS) determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968) [10]. The leaf tissues were homogenized in 0.1% (w/v) tricholoacetic acid (TCA). The homogenate was centrifuged at 10,000 g for 20 min. To l ml of the resulting supernatant, 4 ml of TCA (20%) containing (0.5 w/v) of TBA were added. The mixture was heated at 95 0C for 30 min and then cooled in ice followed by centrifugation at 10,000 g for 15 min. The absorbance was measured at 532 nm and corrected for 600 nm. The concentration of TBARS was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

For proline estimation the primary leaves (1 g) were homogenized with 3% aqueous sulfosalicylic acid and centrifuged at 3,000 g for 10 min. Proline from the supernatant was determined calorimetrically with toluol, accordance the method of Bates et al. (1973) [3].

The leaf gas exchange elements were determined by means of a portable infrared gas analyzer LCA-4 (Analytical Development Company Ltd., Hddesdon, England) equipped with a PLCB-4 chamber.

Relative water content (RWC) was calculated according to Morgan (1986) [22]. Whole leaf or leaf disks (1.5 cm) were weighed immediately after collection or punching (fresh weight, FM) and placed in a Petri dish containing wet filter paper and kept at 4oC in the dark. After 24 h, the turgor weight (TW) was obtained. For the dry weight (DM), leaf disks were oven-dried for 24 h at 80-90°C and weighed.

The water potential (Ψ) in leaves was measured with a pressure chamber (ELE-International, England).

The leaf area (electronic area meter – NEO-3, TU-Sofia) was measured. The stomata cells were measured by means of a bolt eye-lens - micrometer 15x and an objective 15x, at magnification 600x. The stomata cells' photographs were taken by means of an eye-lens 16x and an objective 40x, at magnification 640x.

Statistical analysis

All analyses were done in five replications. The shown results are mean values ± SE. Significant differences between control and each treatment was determined by the Student's " t" criterion.

RESULTS AND DISCUSSION

The effect of two salt stresses on photosynthesis is shown in Table 1. The g_s decreased as a result of submitting to NaCl or Na_2SO_4, but the degree of inhibition varied greatly between the salt sources. Na_2SO_4 decreased it to a greater extent (62 % decreased) than NaCl (50 % inhibition). A significant inhibition in Pn by leaves subjected to both salt treatments was observed, trough there was no striking difference in the magnitude of inhibition between the salinity source (62% decrease by NaCl and 66 % by Na_2SO_4).

According to Tezara [26], both water and salinity stress markedly inhibited Pn and g_s although the effect of salinity on these variables was milder than water deficit.

The results in the same table indicate as well that E was inhibited to e lesser degree in comparison to Pn (58 % decrease by NaCl and 65 % by Na_2SO_4), and once more, the Na_2SO_4 inhibiting effect was more strongly expressed.

The water use efficiency (P_N /E) was modified as a result of the reported changes in P_N and E. In the leaves of beans, subjected to 100 mM NaCl and Na_2SO_4, the P_N /E were reduced to 27-32% compared to that in the control

Table 3. Effect of salinity on the parameters of water exchange in bean plants.
Young bean plants are subjected to salt stress by adding 100 mM of NaCl and Na_2SO_4 to the Hoagland nutrient solution. WD - Water deficit (%), RWC - Relative water content (%), Ψ - Water potential [-MPa] and P – proline content (µg g^{-1} FM). Values are mean ±SE. * <0.5 ** <0.1 ***< 0.01

Таблица 3. Ефект от засоляването върху водния статус на фасула. . WD – вВден дефицит (%), RWC – относително водно съдържание (%), Ψ – Воден потенциал [-MPa] и P – съдържание на пролин (µg g^{-1} FM). Стойностите са осреднени ±SE. * <0.5 ** <0.1 ***< 0.01

Parameters	Control	100 mM NaCL	100 mM Na_2SO_4
WD	13.72±0.25	32.21±0.18**	37.86±0.15***
RWC	86.27±0.28	75.63±0.35	72.53±0.32*
Ψ	2.01±0.07	3.96±0.06**	4.77±0.18***
Proline	25.42±0.53	30.50±0.36*	75.43±0.40**

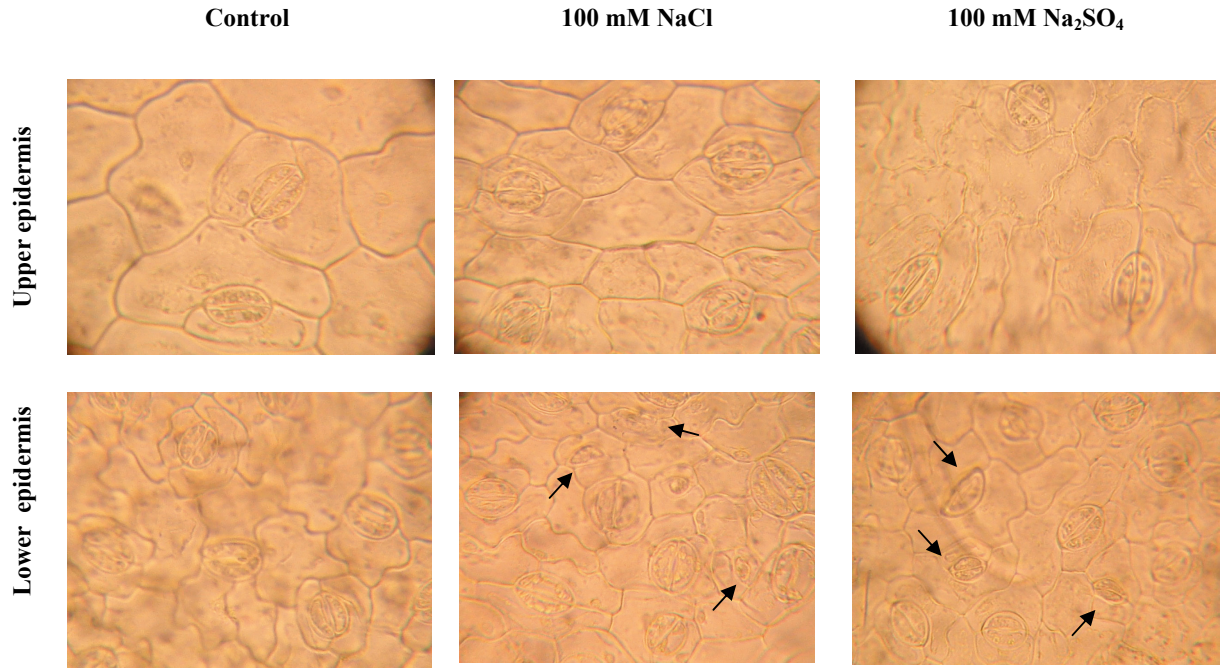

Figure 1. The effect of salinity on stomata frequency.
Снимка 1.Ефект от засоляването върху броя на устицата

plants.

A similar tendency was observed with respect to the changes in the synthesized leaf area (same table).

The decline in photosynthesis observed in case of salinity could be attributed to stomata factors. Stomata closure was induced by the presence of sodium ions in the apoplast surrounding the quard cells [2]. During salt stress the concentration of CO_2 in chloroplasts decreased as a result of the reduction in stomata conductance, in spite of the apparent stability of CO_2 concentration in intercellular spaces [28]. Brougnoly and Lauteri [4] also indicated that reduced photosynthetic carbon assimilation was attributed to reduced stomata conductance.

Since transpiration rate followed the same trend as that in photosynthesis, it is clear that a reduction in photosynthesis has same effects on both stomata and transpiration as the three are integral elements of the photosynthetic apparatus of plants [9]. Many studies have reported that both stomata and non-stomata components are responsible for a decrease in Pn [27]. It was also observed that the stomata conductance of plants varied of salinity types .

The results in Table 2 show leaf anatomical parameters changed with higher external salinity. Salinity of 100

mM NaCl and Na_2SO_4 caused a increase in the stomata frequency at about 42% related to the control plants (Figure 1). The increased stomata frequency at higher salinity agrees with the observations in beans (30). This could be explained with the dwindling of the leaf cells as a result of the xeromorphic structure of the salt-treated plants. The results show that the stomata size (length and width) in salt-treated plants were reduced too.

Some authors have considered the reduction in cell size under water stress to be drought adaptation mechanism [23]. According to Culter et al., [6] the reduction in sell size appears to be a major response of cells to water deficiency that may be caused either by drought or salinity stress. Carimi et al., [5] confirmed, as well, the decrease of the stomata size in salt-treated plants. Water status is highly sensitive to salinity and therefore is dominant in determining the plant responses to stress [26].

Table 3 shows the changes in RWC and water potential in young bean plants under various salinity stresses. After 7 days of treatment the osmotic potential of leaves decreased under saline conditions – 98 % (100 mM NaCL) and 138 % (100 mM Na_2SO_4). Generally, Ψ decreased lower in the second salt. The treatment of plants with Na_2SO_4 causes more considerable changes with respect and to RWC – 14 % lower than the non-treated plants. Dehidratation symptoms were more evident in the 100 mM Na_2SO_4, due to the fact that probably this salt has higher sodium concentrations (2 Na^+) which increased cellular water loss. The decrease of RWC and Ψ leads to an increase of the proline content – 290 % in case of 100 mM Na_2SO_4 treatment. The considerable decrease of RWC and Ψ probably is a result of the structural-functional changes, ensuring the plants adaptation to the salt-treatment. In such cases, a process of osmotic self-adjustment occurs in the plant cells, directed towards the preservation of the water balance by means of accumulation of osmotically active solutes [29], and that is a mechanism reported to operate in plants in response to salt stress [27]. The results from our study with respect to the considerable praline increase in bean leaves coordinate well with the results comcerning the changes in the other two parameters - RWC and Ψ. According toCarimi et al., [5] proline is accumulated in the cytoplasm and its organelles in order to maintain photosynthesis.

Peroxidation of membrane lipids is an indication of membrane damage and leakage under salt stress conditions. Bean plants were affected by both organs by means of lipid peroxidation, but in the roots the MDA content was greater. The results in Figure 2 show as well, that the MDA increased more significantly in the second salt 100 mM Na_2SO_4 (155 % in shoots and 184 % in roots). Lipid peroxidation is often used as an indicator of salt-induced oxidative stress in sells, by the increase in MDA level [8].

On the basis of the results obtained during the research, the following conclusion can be drawn:

The applied doses of both salt types caused stress in the young bean plants, which found expression in the suppression of photosynthesis activity and caused changes in stomata status (conductivity, number and size), which is a result of the disturbed osmotic processes and the toxic effect of Cl^- , SO_4^{2-} and Na^+. The transpiration and the cell water potential in salt-treated plants were

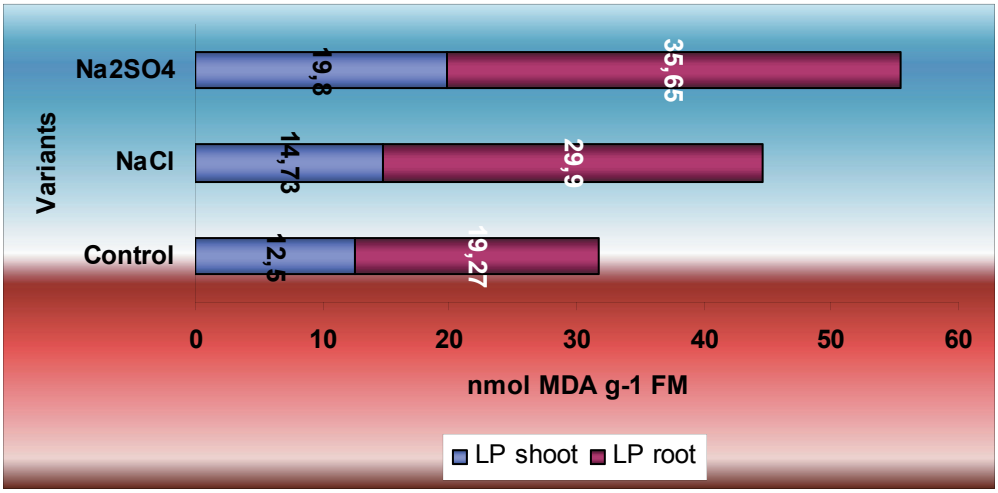

Figure 2. Effect of salinity on lipid peroxidation [LP-[nmol (MDA) g-1 (FM)] in shoots and roots in bean plants.
Фигура 1. Ефект отзасоляването върху нивото на липидната пероксидация [LP-[nmol (MDA) g-1 (FM)] в стъблата и корените на фасула.

reduced and this tendency was more strongly expressed in the case of the sulphate treatment. The proline and MDA content was increased significantly.

With respect to all indexes, a stronger toxic effect of Na_2SO_4, compared to that of NaCl, was registered, a fact probably due to the higher concentration of Na^+ in the bean plants tissues.

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